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EFFECT OF LENGTH OF INDUCTION PERIOD ON FLORAL DEVELOPMENT OF XANTHIUM PENNSYLVANICUM

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR
OF PHILOSOPHY

DEPARTMENT OF BOTANY
1940

By
FRANCES LLOYD NAYLOR

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CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 528

FRANCES LLOYD NAYLOR

(WITH FOURTEEN FIGURES)

Introduction

Xanthium pennsylvanicum has been classified as a short-day plant. It tends to produce only vegetative growth on 24-hour cycles consisting of more than 16 hours of light and less than 8 hours of darkness and tends to flower when the photoperiods are shorter than $15\frac{1}{2}$ hours with accompanying dark periods of more than $8\frac{1}{2}$ hours. If exposed to a few cyclic alternations of light and darkness favorable for flowering, a plant may subsequently flower even though transferred to cycles which with continuous treatment would tend to result in purely vegetative activity. HAMNER (3) has termed these cyclic alternations of light and darkness which tend to induce flowering "photoinductive cycles." With *Xanthium* the so-called short day would constitute a photoinductive cycle; but, as he has shown, other cycles may induce floral initiation even though such cycles could not properly be considered under the term short day. In *Xanthium* a photoinductive cycle contains a specific minimum photoperiod of definite minimum intensity followed by a continuous dark period of more than 9 hours (3).

NEIDLE (5) and HAMNER (3) have found that the rate and nature of the development of the inflorescences of *Xanthium* are affected by the environmental conditions subsequent to the induction period. The former studied the effects of nitrogen supply and the latter primarily the effects of various conditions of light during the postinductive period.

It has been shown (4) in *Xanthium* that one photoinductive cycle may result in subsequent initiation of floral primordia. Under such conditions the rate of development of these primordia into mature flowers and fruits may be very slow. Since treatment with more than one photoinductive cycle seems to affect the rate of floral development, and since it has been shown with Biloxi soybean (1) also that the rate of floral development and the number of flowers initiated are affected by the number of photoinductive cycles to which the plants are exposed, an anatomical study of the floral development in plants which had been exposed to various numbers of photoinductive cycles was made.

Procedure

The experiments reported here were conducted in the summer of 1939. *Xanthium* plants were planted on August 1 in sandy garden soil in clay pots and grown in the greenhouse. During the first 25 days all plants were grown under conditions of long photoperiod, being exposed to supplementary illumination from dusk until midnight. This illumination was obtained from 200-watt Mazda filament lamps and supplied about 80 foot candles at the surface of the leaves. Subsequent to the twenty-fifth day the plants were exposed to the various experimental treatments. In these treatments continuous illumination was obtained by burning the lamps from dusk until sunrise. Photoinductive cycles (short photoperiods) were produced by placing the plants on trucks and moving them into dark compartments at 4:00 P.M., where they remained until 8:00 A.M. the following morning. From then until 4:00 P.M. the trucks stood in the lighted greenhouse.

On the twenty-fifth day the terminal buds of some of the plants were removed and preserved for subsequent examination. All such buds proved to be strictly vegetative, and the assumption is made that all comparable plants were vegetative at this time. As a further check, certain of the plants were continued for 4 more days under long-photoperiod conditions on the greenhouse bench, and samples were preserved and examined histologically. These were also strictly vegetative. The remaining plants were started on the various experimental treatments after the twenty-fifth day (August 25). One lot of 320 plants was exposed continuously to photoinductive cycles, and terminal buds of ten plants were collected each day for 32 days and preserved for histological examination. These plants of lot 1 were used as a basis of comparison. The second lot, containing 50 plants, was transferred to continuous illumination; the third lot of 100 plants was exposed to one photoinductive cycle; a fourth lot of 100 plants, to four photoinductive cycles; and a fifth lot of 100 plants, to eight photoinductive cycles. Lots 3–5, inclusive, were placed under continuous illumination subsequent to their photoinductive treatment. The terminal nodes of ten plants of each of lots 2, 3, 4, and 5 were collected at weekly intervals for 10 weeks and preserved for histological examination, the first collection being made September 2.

The specimens were preserved in formalin-acetic-alcohol and handled according to the butyl-alcohol paraffin method. Serial longitudinal and transverse sections were cut at 15 μ and stained with safranin, gentian violet, and orange G.

Observations

Control plants

The terminal buds of the plants collected on August 25 were strictly vegetative, as were those from the same bench collected on August 29. The plants of lot 2, which were transferred on August 25 to continuous illumination, were all typically

vegetative at each sampling (fig. 1). From these results it is assumed that all plants were vegetative on August 25, and that plants of groups 3, 4, and 5 which developed floral primordia and flowers while growing in continuous illumination did so because of previous photoinductive treatment.

Continuous photoinductive treatment (lot 1)

The terminal buds of plants collected on the first and second days after the beginning of photoinductive treatments showed no noticeable change from the vegetative condition, but on the third day the terminal growing point was broad, smooth, somewhat elevated, and hemispherical (fig. 2). This was the primordium of the terminal staminate inflorescence. By the fourth day the primordia of the involucral bracts appeared at the base of the terminal staminate inflorescence. In the axils of the young leaves, just below the staminate inflorescence, there appeared the primordia of the carpellate inflorescences (fig. 3). By the fifth day the first flower primordia of the staminate inflorescence were noticeable and the carpellate inflorescence primordia had enlarged to about 0.15 mm. (fig. 4). From this point on the development of the staminate and carpellate inflorescences is described separately.

Staminate inflorescence.—The flower primordia and the primordia of the floral bracts developed in spiral acropetalous succession in the staminate inflorescence and were numerous by the sixth day (fig. 5). The primordia of the central flowers had flattened out and the primordia of the marginal flowers had not only flattened but the initials of the corolla were present at their margins. By the seventh (fig. 6) and eighth days (fig. 7) the inflorescence was about 1.3 mm. in diameter, and the slender peduncle by which the terminal staminate inflorescence is raised above the carpellate inflorescences had elongated, its length being about equal to the diameter of the staminate inflorescence. The primordia of the corolla were present in the central flowers, and in the marginal flowers the corolla primordia and also the stamen primordia were present, the latter arising adaxial to the corolla. Each flower was in the axil of a bract.

At this time one or two small staminate inflorescences (about 0.26 mm. in diameter) were noted within the terminal staminate inflorescence, each small one appearing in the axil of an involucral bract of the terminal inflorescence. Flower and floral bract primordia were present in the small infloresences.

On the ninth day (fig. 10) the terminal staminate inflorescence was 1.8 mm. in diameter. Each flower had a marginal sympetalous corolla, a column composed of five or six undiverged stamens, and the primordia of two undiverged carpels in the center. In some flowers a cleft had appeared at the apex of the carpel primordia. The calyx was absent. By the thirteenth day (fig. 12) each stamen was differentiated into filament and anther. Pollen was shed about the eighteenth day after the

beginning of induction. The rudimentary pistil of each staminate flower consisted of a style with a bifid stigma. The period during which pollen was shed extended beyond the time of shedding from the terminal inflorescence, since the flowers of

FIGS. 1–14.—Terminal region of *Xanthium* represented in each figure. Fig. 1, vegetative plant. Fig. 2, third-day stage of plant which had received continuous photoinductive treatment (lot 1). Fig. 3, fourth day of same. Fig. 4, fifth day. Fig. 5, sixth day. Fig. 6, seventh day. Fig. 7, eighth day. Fig. 8, eighth-day stage of plant which had received one photoinductive cycle followed by continuous illumination (lot 3). Fig. 9, eighth-day stage of plant which had received four photoinductive cycles. Fig. 10, ninth-day stage of lot 1. Fig. 11, eleventh-day stage of lot 1. Fig. 12, thirteenth day of same. Fig. 13, thirty-sixth-day stage of lot 3. Fig. 14, sixty-fourth-day stage of lot 3. (*car*, carpel; *cla*, corolla; *ch*, carpellate inflorescence; *f*, floral bracts; *fp*, flower primordium; *i*, involucral bract; *l*, leaf; *lp*, leaf primordium; *mmc*, microspore mother cell; *o*, ovule; *po*, pollen; *s*, spine; *sta*, stamens; *sti*, stigma; *sth*, staminate inflorescence; *p*, perianth; *sty*, style.)

the small inflorescences in the axils of the involucral bracts developed more slowly than did the flowers of the terminal inflorescence itself.

CARPELLATE INFLORESCENCE.—The primordia of the involucral bracts were

present at the base of the primordium of the topmost carpellate inflorescence by the sixth day (fig. 5). By the seventh day the inflorescence was 0.6 mm. in diameter and many protuberances had appeared on the surface. The lower ones, about fifteen in all, were the primordia of the involucral bracts; the upper ones were the primordia of the spines. The receptacle of the carpellate inflorescence was flattened at the apex (fig. 6). The margins of the receptacle, and the tissue at the exact center between the margins, became slightly elevated. Continued growth of these regions resulted in the formation of two depressions, which became wide at the bottom and narrow above. On one side the margin grew more rapidly and thus one depression became deeper than the other.

By the eighth day flower primordia appeared simultaneously in the bottom of each depression. The flower in the lower depression developed somewhat more rapidly and became larger than the other. The central tissue, which was a partition between the two depressions, developed a cleft in its upper surface (fig. 7). By the ninth day (fig. 10) the primordium of the perianth was evident at the margin of each flower primordium. By the eleventh day the two carpels had appeared in the center of the flower (fig. 11). The upward growth of the carpels formed the single cavity of the ovary. The carpels grew much more rapidly than did the perianth, and by the thirteenth day (fig. 12) the perianth appeared as a small flange on the outer margin of the carpels. The carpels continued elongation and formed a long style and a bifid stigma. About the twelfth day an ovule began developing in each ovary.

All flower parts were present by the thirteenth day, and further development consisted of increase in length and breadth. The megagametophyte was mature by the seventeenth day. The margin and central tissue of the receptacle grew far above the flowers. One part of the central tissue, with half the margin, formed the beak above one flower. The other half of the central tissue and the other half of the margin formed the beak above the second flower.

The carpellate inflorescences in the axils of the third and fourth leaves below the terminal staminate inflorescence developed more rapidly and became larger than did the uppermost carpellate inflorescences. For example, on the eighth day the flower primordia were present in the depressions of the uppermost inflorescences, but in the lower inflorescences the perianth and carpel primordia were already differentiated. At the end of a month the seed of the uppermost bur was about 3.5 mm. long and the embryo about 1.5 mm. long. The lower bur contained a seed 6.5 mm. long and an embryo 2.5 mm. long.

FARR (2) has stated that the bur is a modified capitulum and that it differs from the typical capitulum of the Compositae only in having two depressions in the receptacle. He believes that the spines are specialized bracts and the beaks are portions of the receptacle.

Various inductive treatments (lots 3–5)

1. One photoinductive cycle.—The inflorescences and flowers of these plants were greatly delayed in development as compared with those which received continuous photoinductive treatment (lot 1). By the eighth day (fig. 8) after induction the staminate inflorescence showed the primordia of the flowers and leaf bracts, but no flower parts had differentiated. The primordia of the involucral bracts of the inflorescences in the axils of the young leaves were just visible. This stage was shown on the fifth day by the plants of lot 1. By the fifteenth day the marginal flower primordia of the terminal staminate inflorescence showed flattened apices. The floral bracts were clearly distinguishable from the flower primordia, a stage comparable with that of the plants of lot 1 on the sixth day. The axillary inflorescences showed little advance in development, the inflorescences being somewhat cone-shaped, the involucral bracts differentiated, but the type of inflorescence not yet distinguishable.

By the twenty-second day the marginal flowers of the terminal staminate inflorescence had developed the primordium of the corolla and a few of the central flowers had flattened apices, a stage comparable with the plants of lot 1 on the seventh day. The axillary inflorescences showed no change in development. By the twenty-ninth day the marginal flowers of the terminal staminate inflorescence resembled those of lot 1 on the eighth day, having the corolla, stamen, and carpel primordia established; but the most central flowers were just beginning to show corolla primordia. There was a greater difference in the size and stage of development between the outer and inner flowers of this inflorescence than in those of lot 1. This difference became increasingly marked as the plants grew older. The peduncle by which the terminal staminate inflorescence is raised above the topmost leaves was longer than in the plants of lot 1.

In the terminal staminate inflorescence there were one or two axes, each appearing in the axil of an involucral bract of the terminal staminate inflorescence. Each axis was composed of one small terminal and two small lateral staminate inflorescences, rather than of a single staminate inflorescence as in lot 1. These small inflorescences had flower primordia and bracts but no flower parts differentiated. Unlike the small staminate inflorescences of lot 1, these were elongated. The internodes of each small inflorescence had continued longitudinal growth and the individual flowers developed one above the other on the axis in the axils of the floral bracts. By the thirty-sixth day the small staminate inflorescences had increased in size, and by the forty-third day the floral bracts were very long. It seems probable that these floral bracts may become the leafy organs often noted on staminate inflorescences of *Xanthium* plants which have received a few induction periods and then been grown on long photoperiod for several months (5).

An individual carpellate inflorescence or an axis consisting of three carpellate

inflorescences usually appeared in the axils of the first, second, third, and fourth leaves below the terminal staminate inflorescence in the plants of lot 1. In some of the plants which received only one photoinductive cycle (lot 3) a lateral axis consisting of three small staminate inflorescences appeared in the axil of the topmost leaf. These inflorescences were elongated rather than round. In the terminal inflorescence of this lateral axis flower primordia and floral bracts were present. In the axils of other upper leaves there were carpellate inflorescences. In the axils of leaves below these the primordia were so slightly developed that their type could not be determined.

By the thirty-sixth day all the flower parts had formed in the marginal flowers in the terminal staminate inflorescence, the largest staminate inflorescence of the plant. The central flowers, however, were still in the primordial stage (fig. 13). By the sixty-fourth day nearly all the central flowers of the terminal staminate inflorescence had formed corolla and stamen primordia (fig. 14). Carpellate inflorescences in which the depressions were beginning to form were found in the axils of some of the upper leaves of the plant. The inflorescences did not develop further before the end of the experimental period.

2. Four photoinductive cycles.—These plants had received four photoinductive cycles (four short photoperiods) and 4 days of continuous illumination when the first samples were taken at the end of the eighth day (fig. 9). In the terminal staminate inflorescence the individual flowers were nearly as well developed as were those of lot 1. The one or two small staminate inflorescences which appeared in the axils of involucral bracts of the terminal staminate inflorescence were about three times as large as those on the plants of lot 1, being about 0.8 mm. in diameter. Their flower and bract primordia could be distinguished, but no flower parts had been differentiated. The staminate inflorescences were round, not elongated like the small ones on the plants which had received one cycle of induction. Subsequent development was somewhat delayed, a stage comparable with that of lot 1 on the eleventh day being reached by the fifteenth day. The first pollen was shed about 3 weeks after the shedding by plants of lot 1. The megagametophytes were mature by the thirty-sixth day. The inflorescences in the axils of the involucral bracts formed their flower parts later than did the terminal inflorescence itself.

3. Eight photoinductive cycles.—At the time of the first collection of lot 5, 8 days after the beginning of the photoinductive treatments, these plants had not yet been moved to continuous illumination. They were in the same stage of development as were the plants of lot 1 on the eighth day. Samples from later collections, taken after these plants had been moved to continuous illumination, showed that development (as compared with lot 1) was only slightly delayed. By the twenty-second day the megagametophytes were mature. Pollen was shed about 5 days after the shedding by the plants of lot 1.

The number of staminate and carpellate inflorescences in the upper five nodes of

plants which had received one, four, and eight photoinductive cycles was compared with the number on the plants under continuous photoinductive treatment (lot 1). Stages from each group, comparable with the thirteenth day of the latter (the eighty-fourth-day stage of the plants induced one day, etc.), were selected because at this stage the character of the inflorescences could best be determined.

On the plants of lot 1 there were present a terminal staminate inflorescence and also two small staminate inflorescences, each in the axil of an involucral bract of the terminal inflorescence. The inflorescences in the axils of the four topmost leaves were carpellate. In some plants the axis in the axil of the fifth leaf below the terminal staminate inflorescence bore lateral carpellate inflorescences and terminated in a staminate inflorescence. There were three or four staminate and twelve or thirteen carpellate inflorescences per plant at the upper five nodes.

On the plants which received one photoinductive cycle the main axis terminated in a staminate inflorescence. Two lateral axes, each in the axil of an involucral bract of the terminal inflorescence, were each composed of three small staminate inflorescences. In the axil of the topmost leaf of some plants there were three staminate inflorescences; those in the axils of two other upper leaves were carpellate. In many plants the axes in the axils of the fourth and fifth leaves below the terminal staminate inflorescence were so slightly differentiated that it was impossible to tell whether they were vegetative or floral. There were about ten staminate and six carpellate inflorescences in the upper five nodes.

The plants which received four photoinductive cycles and the ones which received eight had about the same number of staminate and carpellate inflorescences as had the plants which received continuous photoinductive cycles (lot 1).

Summary

1. Histological examinations were made of the developing carpellate and staminate inflorescences of *Xanthium pennsylvanicum*. The plants were exposed after the twenty-fifth day of vegetative growth to (*a*) continuous photoinductive cycles, (*b*) one photoinductive cycle, (*c*) four photoinductive cycles, (*d*) eight photoinductive cycles. The study included those inflorescences which developed from the terminal bud and the buds in the axils of the five uppermost leaves.

2. Development of both carpellate and staminate inflorescences in the plants exposed continuously to photoinductive cycles was more rapid than that in any other group. All flower parts were present by the thirteenth day, and at the end of the month the seeds were almost mature.

3. Development of inflorescences in the plants which received only one photoinductive cycle was much slower than that of plants which received continuous photoinductive treatment. The flower parts of the former did not appear in all the flowers of the terminal staminate inflorescence until the sixty-fourth day after induction, rather than by the thirteenth day as in the latter. The former also dif-

fered from the latter in that there was a greater difference in the size and stage of development between the outer and inner flowers of the inflorescence.

4. The terminal staminate inflorescence of the plants receiving one photoinductive cycle, as well as of the plants exposed continuously to photoinductive treatment, contained one or two axes, each appearing in the axil of an involucral bract of the terminal inflorescence. Each axis of the former was composed of three staminate inflorescences, rather than of a single one as in the latter. The small staminate inflorescences of the former were much more elongated than those of the latter.

5. In the axil of the uppermost leaf a lateral axis consisting of three staminate inflorescences appeared in some of the plants exposed to one photoinductive cycle, while in those exposed continuously to photoinductive treatment an individual carpellate inflorescence or an axis consisting of three usually occupied this position. The staminate inflorescences of the former were elongated rather than round.

6. In the plants exposed to one photoinductive cycle there developed approximately ten staminate and six carpellate inflorescences, while in the plants exposed continuously to photoinductive cycles there was an average of about four staminate and twelve or thirteen carpellate inflorescences per plant. Thus, increasing the number of induction treatments seemed to stimulate the production of carpellate more than of staminate inflorescences.

7. The plants which received four photoinductive cycles exhibited, in the axils of involucral bracts of the terminal staminate inflorescence, one or two staminate inflorescences three times as large as those occupying this position in plants exposed continuously to photoinductive treatment.

8. The development of the inflorescences on those plants which received four or eight photoinductive cycles resembled more closely the development in plants continuously exposed to such treatment than that in plants exposed to one photoinductive cycle. The rate of development was intermediate between the two.

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Department of Botany
University of Chicago

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